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1. (Twice amended) A method of counting a single copy of a target species immobilized on a substrate, said method comprising:

(i) detecting a single copy of said target species by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate, and further wherein said first quantum dot is distinguishable from said second quantum dot, thereby counting said single copy.

2. (Once amended) The method according to claim 1, wherein said first quantum dot and said second quantum dot are attached to said target species prior to binding said target species to said affinity moiety.

3. (Once amended) The method according to claim 1, wherein said first quantum dot and said second quantum dot are attached to said target species after binding said target species to said affinity moiety.

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5. (Once amended) The method according to claim 1, wherein binding of said target species to said affinity moiety forms a target species-affinity moiety complex that is detected by fluorescence from both said first quantum dot and said second quantum dot attached to said target species-affinity moiety complex.

6. (Once amended) The method according to claim 1, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

7. (Once amended) The method according to claim 1, wherein said first quantum dot and said second quantum dot are visually distinguishable as a first color and a second color, respectively.

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10. (Twice amended) The method according to claim 1, wherein said first quantum dot and said second quantum dot are attached to a targeting moiety for said target species, said targeting moiety being a member selected from the group consisting of antibodies, aptamers, proteins, streptavidin, nucleic acids and biotin.

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22. (Twice amended) The method according to claim 19, wherein said alignment moiety identifies the position of one or more target moiety-affinity complexes.

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29. (Once amended) A method of counting a single copy of a target species in solution, said method comprising

(i) detecting a single copy of said target species by detecting essentially simultaneously an optical characteristic of a first quantum dot of a first color attached to said single copy and a second quantum dot of a second color attached to said single copy, wherein said first color and said second color are distinguishably different colors, thereby counting said single copy.

30. (Twice amended) A method of counting a single copy of a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate with spacing between each member of said population, said method comprising:

(i) detecting a single copy of said target species by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate, wherein said first quantum dot is distinguishable from said second quantum dot, and further wherein said detecting is performed with a detecting means having a resolution that is higher than said spacing between each member of said population, thereby counting said single copy.

31. (Twice amended) A method of counting a single copy of a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate, said method comprising:

(i) detecting a single copy of said target species by detecting an optical characteristic of a quantum dot attached to said single copy, wherein said first quantum dot is distinguishable from said second quantum dot, and further wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate forming a target-affinity moiety complex, and said detecting is performed with a detecting means having a resolution limited region of interest whereby, less than one target-affinity moiety complex is present within each resolution limited region of interest, thereby counting said single copy.

32. (Twice amended) A method of counting a single copy of a first target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate, said method comprising:

(a) defining a first region of interest of said substrate; and

(b) probing said first region of interest for an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy of said first target species bound to an affinity moiety for said first target species immobilized on said substrate, wherein said first quantum dot is distinguishable from said second quantum dot, thereby counting said first target species.

33. (Twice amended) The method according to claim 32, further comprising counting a single copy of a second target species immobilized to said substrate, said method comprising:

(c) defining a second region of interest of said substrate; and

(d) probing said second region of interest for an optical characteristic of a third quantum dot and a fourth quantum dot attached to said single copy of said second target species bound to an affinity moiety for said second target species immobilized on said substrate, wherein said third quantum dot is distinguishable from said fourth quantum dot, thereby counting said second target species.

37. (Once amended) A method for counting multiple target species immobilized on a substrate, which species are members of a population of target species immobilized on said substrate, said method comprising:

(a) defining multiple regions of interest on said substrate; and

(b) probing said multiple regions of interest for an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of said target species bound to an affinity moiety for said target species immobilized within a region of interest of said substrate, thereby counting multiple target species.

40. (New) The method according to claim 1, wherein said optical characteristic is detected by coincidence detection.

41. (New) The method according to claim 1, wherein said optical characteristic is fluorescence.

42. (New) The method according to claim 29, wherein said optical characteristic is fluorescence.

43. (New) The method according to claim 31, wherein said optical characteristic is fluorescence.

44. (New) The method according to claim 32, wherein said optical characteristic is fluorescence.

45. (New) The method according to claim 33, wherein said optical characteristic is fluorescence.

46. (New) The method according to claim 37, wherein said optical characteristic is fluorescence.

47. (New) The method according to claim 1, further comprising

(ii) resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single copy from an optical characteristic arising from a quantum dot not attached to said single copy.

48. (New) The method according to claim 29, further comprising

(ii) resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single copy from an optical characteristic of a quantum dot not attached to said single copy.

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49. (New) The method according to claim 32, further wherein said probing resolves said optical characteristic of said first quantum dot and said second quantum dot from an optical characteristic of other members of said population of target species immobilized on said substrate.

50. (New) The method according to claim 33, further wherein said probing resolves said optical characteristic of said third quantum dot and said fourth quantum dot from an optical characteristic of other members of said population of target species immobilized on said substrate.

51. (New) The method according to claim 37, wherein said probing resolves the optical characteristic of said first quantum dot and said second quantum dot from other members of said population and from each other.

REMARKS

The Invention

The present invention relates to methods of detecting the presence of a single copy of a target species in a sample by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to the single copy of the target species. The first quantum dot and the second quantum dot are distinguishable. In some embodiments of the invention, the first quantum dot and the second quantum dot are visually distinguishable as a first color and a second color. In some embodiments of the present invention, the first quantum dot and the second quantum dot combine to form a color that is distinguishable from the color of the first quantum dot and the color of the second quantum dot.